Biomarkers to predict the response to cardiac resynchronization therapy

Heggermont, Ward ; Auricchio, Angelo ; Vanderheyden, Marc

Abstract: Cardiac resynchronization therapy (CRT) is an established non-pharmacological treatment for selected heart failure patients with wide QRS duration. However, there is a persistent number of non-responders throughout. The prediction of the CRT response is paramount to adequately select the correct patients for CRT. One of the expanding fields of research is the development of biomarkers that predict the response to CRT. A review of the available literature on biomarkers in CRT patients has been performed to formulate a critical appraisal of the available data. The main conclusion of our review is that biomarker research in this patient population is very fragmented and broad. This results in the use of non-uniform endpoints to define the CRT response, which precludes an in-depth comparison of the available data. To improve research development in this field, a uniform definition of the CRT response and relevant endpoints is necessary to better predict the CRT response.

DOI: https://doi.org/10.1093/europace/euz168

ESC European Society of Cardiology

Europace (2019) **0**, 1–12
doi:10.1093/europace/euz168

REVIEW

Biomarkers to predict the response to cardiac resynchronization therapy

Ward Heggermont [1,2]*, Angelo Auricchio[3,4], and Marc Vanderheyden[1]

[1]Cardiovascular Research Centre, OLV Hospital Aalst, Moorselbaan 164, B-9300, Aalst, Belgium; [2]Cardiovascular Research Institute Maastricht, Maastricht University, Universiteitssingel 50, 6229, Maastricht, The Netherlands; [3]Cardiocentro Ticino, Department of Electrophysiology, Via Tesserete 48, CH-6900, Lugano, Switzerland; and [4]Centre for Computational Medicine in Cardiology, Via Buffi 13, CH-6900, Lugano, Switzerland

Received 21 February 2019; editorial decision 20 May 2019; accepted 23 May 2019

Cardiac resynchronization therapy (CRT) is an established non-pharmacological treatment for selected heart failure patients with wide QRS duration. However, there is a persistent number of non-responders throughout. The prediction of the CRT response is paramount to adequately select the correct patients for CRT. One of the expanding fields of research is the development of biomarkers that predict the response to CRT. A review of the available literature on biomarkers in CRT patients has been performed to formulate a critical appraisal of the available data. The main conclusion of our review is that biomarker research in this patient population is very fragmented and broad. This results in the use of non-uniform endpoints to define the CRT response, which precludes an in-depth comparison of the available data. To improve research development in this field, a uniform definition of the CRT response and relevant endpoints is necessary to better predict the CRT response.

Keywords Heart failure • Cardiac resynchronization therapy • CRT response • CRT non-responder • Cardiac reverse remodelling • Biomarkers

Introduction

Cardiac resynchronization therapy (CRT) is an extensively validated treatment for symptomatic heart failure (HF) patients with reduced left ventricular ejection fraction (LVEF), prolonged QRS duration, and abnormal QRS morphology. Following the most recent European Society of Cardiology (ESC) guidelines,[1] there is strong evidence for CRT implantation in HF patients [New York Heart Association (NYHA Class II–IV)] with a LVEF below 35%, and with a QRS duration of more than 150 ms with left bundle branch (LBB) block morphology. For other subgroups of patients, CRT might still be useful although evidence is less compelling.[1] However, despite a moderate improvement over the last two decades, the response to CRT is still rather mixed. The recent Clinical Trial of the SonRtip lead and automatic AV-VV optimization (RESPOND-CRT) trial[2] showed a clinical composite response rate of 77% in CRT patients with LBB block and only 66% in CRT patients without LBB block. Furthermore, the proportion of so-called 'super-responders' to CRT defined by almost complete normalization of ventricular function and volumes) has remained constant over time, still representing not more than 30% of all CRT patients. The timely prediction of a CRT response, especially a super-response, is therefore of paramount importance. Although numerous other factors do determine the success of CRT (e.g. optimization of the device, lead positioning, etc.), early identification, or even prediction, of CRT response might have long-term beneficial therapeutic consequences.

Numerous attempts have already been made to find a reliable biomarker(s) to predict the response to CRT. A good, clinically useful biomarker has to be specific for the condition that needs to be detected, yet at the same time display adequate sensitivity for the pathologic condition. Ideally, it has strong predictive value, is robust and reproducible over time. Preferentially, the biomarker can be assessed non-invasively, is readily accessible (e.g. a simple blood test) and its use should be supported by both pre-clinical and clinical data.

In this review article, we aimed to present a summary of the studies on biomarkers that have been performed so far, highlighting the fact that more focused research is mandatory to find a reliable biomarker (panel) to predict the CRT response.

Methodology and search strategy

PubMed.gov as a research literature platform (http://ncbi.nlm.nih.gov), and Medical Subject Headings (MeSH) were used to identify relevant publications irrespective of the publication date. The following MeSH terms were combined as follows: 'biomarkers' [MeSH] OR 'microRNA' [MeSH] OR 'protein' [MeSH] OR 'genes' [MeSH] AND

* Corresponding author. Tel: +32 53 72 44 39; Fax: +32 53 72 45 87. E-mail address: ward.heggermont@olvz-aalst.be

'cardiac resynchronization therapy' [MeSH] OR 'cardiac resynchronization therapy devices' [MeSH]. This research retrieved 231 papers that were screened for eligibility. Based on careful review of the abstract, 181 records were excluded. The remaining 50 papers were assessed for eligibility based on full-text review. A total number of 8 papers were excluded, because of the following reasons: 4 were reviews, 1 paper only described a study protocol and no data, and 3 manuscripts were basic research papers (no patient data), irrelevant for this review. Finally, the remaining 42 papers were included in the qualitative analysis of this review. The search was conducted in November 2017 and updated in June 2018.

Cardiac biomarkers in resynchronization therapy

Brain-derived natriuretic peptide (BNP) and its amino-terminal linked counterpart (NT-pro-BNP) are widely accepted diagnostic and prognostic markers in HF patients.[1] Therefore, their potential to predict a response to resynchronization therapy has been investigated. In 2013, Brenyo *et al.* investigated the predictive value of BNP in a subgroup of the landmark Multicenter Automatic Defibrillator Implantation with Cardiac Resynchronization Therapy (MADIT-CRT) trial study, with mildly symptomatic HF.[3,4] Both baseline and 1 year of follow-up BNP levels were assessed in 1197 patients. As expected, elevated baseline BNP was associated with a 68% increased risk of HF or death ($P = 0.007$) in patients allocated to CRT-D as well as in those receiving implantable cardioverter-defibrillator (ICD)—in the ICD-only group, the increased risk of HF or death was 58% ($P = 0.02$). Importantly however, at 1 year of follow-up, patients who received CRT showed significantly greater reductions in BNP levels (26%) compared to ICD-only patients (8% increase, $P = 0.005$ for the difference). Moreover, in the CRT-D group, lower 1-year BNP levels were associated with a significantly lower risk of HF or death, compared to the subgroup where BNP levels remained high. Furthermore, the echocardiographic response to CRT-D was the highest in patients maintaining or attaining low BNP levels at 1 year of follow-up.[3] In the same patient group, elevated baseline and follow-up BNP levels were independent predictors of increased risk for ventricular tachyarrhythmias [ventricular tachycardia/ventricular fibrillation (VT/VF)], whereas reduced BNP levels following CRT-D implantation coincided with a lower incidence of VT/VF during follow-up.[5] Further evidence for the usefulness of BNP as a predictor of the CRT response was obtained in a prospective follow-up study of 267 HF patients with an average LVEF of 25% ± 8% (mean + SD) undergoing CRT-D implantation.[6] Both individually and in combination, baseline high-sensitivity troponin T (hsTnT) and BNP values—in a multivariate Cox regression model including age, NYHA class, LVEF, and QRS duration—were independent predictors of outcome, defined as death or HF hospitalization. A risk category based on the elevation of two, only one, or none of the investigated biomarkers, proved a significant predictor of outcome, with respective hazard ratios (HRs) of 7.34 [95% confidence interval (CI) 2.48–21.69] and 2.50 (95% CI 1.04–6.04) for high- and intermediate risk groups. Also, in a smaller study in which 105 HF patients (68% men, aged 65.4 ± 10.1 years) were followed for BNP levels and inflammatory markers, lower BNP levels were observed in both the objective responders to CRT [defined as a reduction of ≥15% in left ventricular end-systolic volume (LVESV)] and in the subjective response to CRT (defined as an improvement of ≥10 points on the patient-reported Kansas City Cardiomyopathy Questionnaire).[7] Altogether, these studies provide convincing evidence for the use of BNP as a predictor of the response to CRT.

Soluble suppressor of tumorigenicity 2 (sST2) is a protein that is encoded by the IL1R1 gene (interleukin 1 receptor 1) and it basically reflects adverse cardiac remodelling and fibrosis. Its interesting role as a biomarker in HF has been described elsewhere,[8] but its involvement in the CRT response prediction was also assessed in a subpopulation of the MADIT-CRT trial ($n = 410$, NYHA Class I/II). In multivariate-adjusted models, elevated baseline sST2 was associated with an increased risk of death, death or HF, and death or ventricular arrhythmia, even when adjusting for baseline BNP levels.[9] Furthermore, lower baseline sST2 levels coincided with a greater risk reduction with CRT-D ($P = 0.006$). This was confirmed upon measuring sST2 serially over time. Unfortunately, no data are available about serial measurements of sST2 after CRT and how baseline sST2 levels predict the response to CRT.

Galectin-3, also a marker of fibrosis, is a soluble beta-galactoside-binding lectin that has a regulatory function in fibrosis, tissue repair, and inflammation.[10] Again, this biomarker was tested in a subgroup of the MADIT-CRT population ($n = 654$, NYHA I/II)[11] with non-fatal HF events or death as study endpoints. Patients having a baseline galectin-3 level in the upper quartile of the distribution, also had a 65% reduction in the occurrence of the primary endpoint if given CRT-D (HR 0.35, 95% CI 0.19–0.67). In contrast, patients having lower galectin-3 levels at baseline only had a 25% non-significant risk reduction (HR 0.75, 95% CI 0.51–1.11), meaning that the patients with the highest risk for HF had the greatest benefit of CRT implantation. Baseline galectin-3 level was an independent predictor of outcome (HR 1.55, 95% CI 1.01–2.38; $P = 0.043$).[11] Similar results were obtained in a subpopulation of the CARE-HF trial,[12] showing galectin-3 as an independent predictor of worse overall cardiovascular outcome, however not predicting the response to CRT as a separate outcome parameter.[13] Although caution needs to be taken when interpreting all these results together, it seems that sST2 and galectin-3, markers of fibrosis and to some extent ventricular remodelling, better reflect the ongoing processes during successful resynchronization therapy, compared to NT-pro-BNP, BNP, or troponin. However, a head-to-head comparison in a prospective follow-up study would be helpful to determine the most specific cardiac biomarker related to the CRT response.

The abovementioned biomarkers (NT-pro-BNP, hsTnT, galectin-3, and sST-2) were also tested for their predictive value in estimating the improvement of mitral regurgitation (MR) after implanting a CRT device in 132 patients. From the BIOCRT study,[14] it appeared that higher galectin-3 levels at the time of CRT implantation conveyed a MR non-improvement (status quo or worsening) after 6 months. Although these patients also had higher hsTnT at baseline, after multivariate analysis only galectin-3 prevailed as a statistically significant predictor of MR evolution. Conversely, in the patients who had an improvement in MR, absolute levels of NT-pro-BNP and sST2 were lower at follow-up however without reaching statistical significance.[14]

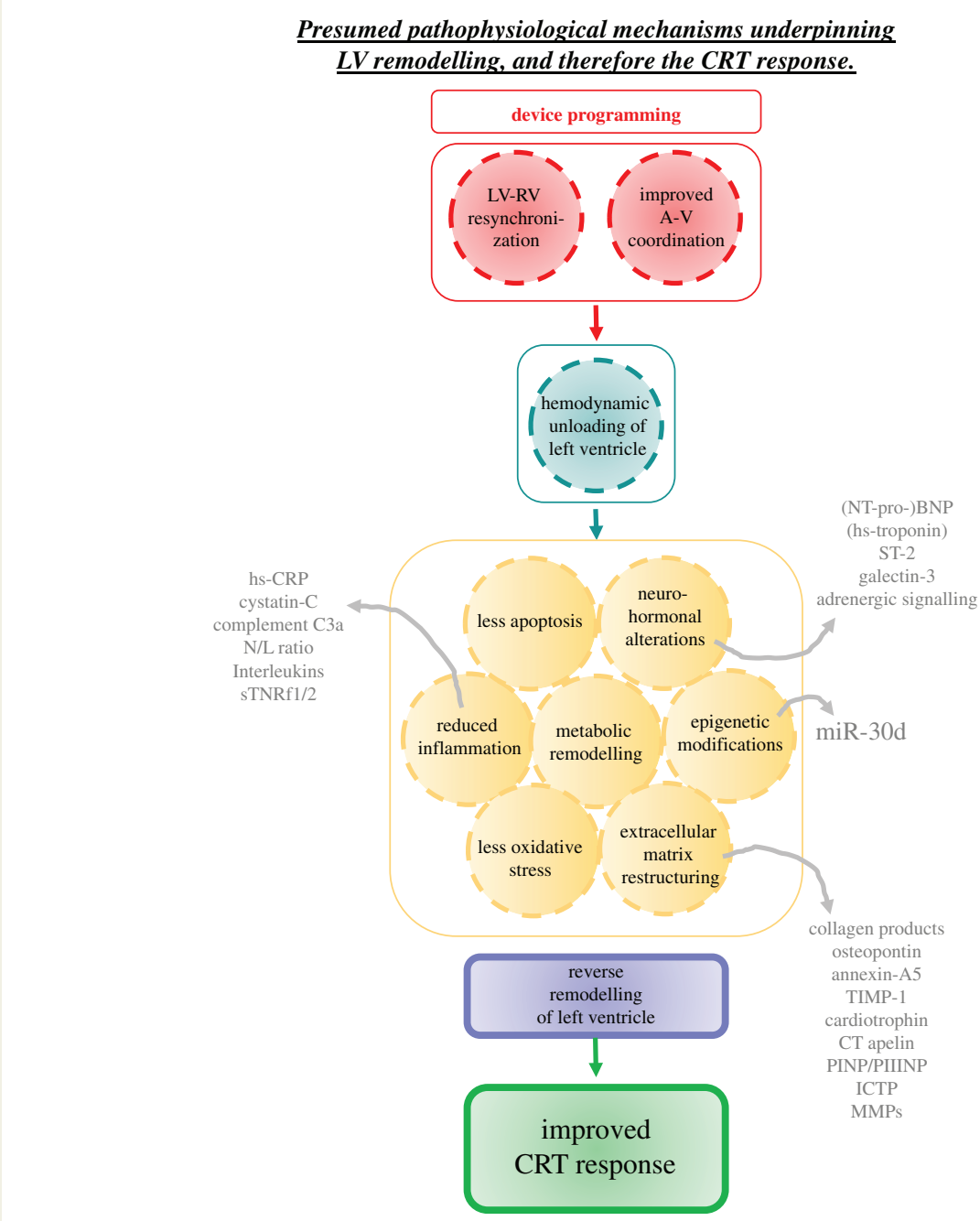

Figure 1 Central figure illustrating the presumed pathophysiologic pathways involved in left ventricular reverse remodelling, and hence the response to cardiac resynchronization therapy. BNP, brain natriuretic peptide; CRT, cardiac resynchronization therapy; CT, carboxyterminal; hs, high-sensitive; LV, left ventricle; miR, microRNA; N/L, neutrophil/lymphocyte; NT, amino-terminal; RV, right ventricle; ST, soluble factor of tumorigenicity; TIMP, tissue inhibitor of metalloproteinase.

Markers of inflammation

(High-sensitivity) C-reactive protein

Heart failure patients display elevated inflammatory markers which correlate with morbidity and mortality.[15] In one study,[16] the predictive power of the most frequently used inflammatory marker, high-sensitivity C-reactive protein (hsCRP) was investigated in 65 HF patients eligible for CRT (46 males, mean age 65 ± 12 years, NYHA III/IV). Levels of both hsCRP and BNP were measured before device implantation. Reverse remodelling, an element of beneficial response to CRT, was defined as >15% reduction in LVESV. In this patient cohort, the hsCRP levels were significantly higher in the non-responders than in the responders ($P < 0.01$). Moreover, multivariate logistic regression analysis showed a relationship between hsCRP

Table 1 Overview of trials investigating CRT response in relation to biomarker levels

References	First author	Publ. year	Number of CRT patients	Biomarker	Definition of CRT response	Time frame (months)	Results	Remarks
3	Brenyo	2013	1197	BNP	Echocardiographic: reduction in LVESV as a continuous variable	12	• Elevated baseline BNP was associated with 68% ($P = 0.007$) (CRT-D) and 58% ($P = 0.02$) (ICD) increase in risk of HF or death • If BNP was low, or reduced, after implantation, CRT response was significantly better	Prognostic but not predictive for response
6	Shalaby	2015	267	BNP Troponin T	Echocardiographic response (not further defined)	12	• Division in three groups: high TnT + high BNP, low TnT + low BNP, or a combination of one high- and one low-level biomarker • Significant difference in event-free survival: the higher the levels of the biomarkers, the worse the survival (log-rank $P < 0.001$)	Prognostic but not predictive for response
7	Brouwers	2014	105	BNP Inflammatory markers	>15% decrease in LVESV	14	• Subjective responders had lower TNF-alpha levels at baseline, but no difference in BNP • Objective response was associated with lower BNP over time • Subjective response was associated with lower TNF-alpha levels	Strongest association between subjective response to CRT, not objective response
16	Kamioka	2012	65	hsCRP	>15% decrease in LVESV	6	Multivariate logistic regression analysis showed an independent relationship between hsCRP and the incidence of non-responders (OR 1.499, $P = 0.011$)	Limited patient number
17	Marin	2011	36	Inflammation ECM elements	echocardiographic Response (not further defined)	3	CRP levels significantly decreased following successful resynchronization	Very limited patient number
18	Szlepaki	2016	126	Complement C3a NT-pro-BNP	Not defined	6	CRT reduced C3a ($P < 0.0001$), sC5b-9 ($P = 0.0006$) but not total C3 levels; and C3a predicted 5 years of mortality of patients independent of NT-pro-BNP	Effect on mortality, not on CRT response
20	Boros	2016	122	Neutrophil/lymphocyte ratio	>15% decrease in LVESV	6	A baseline NL ratio exceeding 2.95 predicted lack of remodelling and 2 years of mortality, independent of NT-pro-BNP	–
21	Belperio	2016	257	Inflammatory mediators	>15% decrease in LVESV	12	Baseline detectable IL-13 was significantly associated with the CRT response, while baseline FGF-2 was negatively associated	–

Continued

Table 1 *Continued*

References	First author	Publ. year	Number of CRT patients	Biomarker	Definition of CRT response	Time frame (months)	Results	Remarks
22	Osmancik	2013	81	Apoptotic and inflammatory markers	'Clinical and echocardiographic improvement'	6	In non-responders, TGF-beta1 levels significantly increased, and baseline TGF-beta1 level was a significant predictor of poor prognosis	–
23	Limongelli	2014	44	Cardiotrophin-1	>15% decrease in LVESV	6.4 ± 0.79	Multivariate logistic model showed CT-1 as an independent predictor of CRT echo response (OR 0.97, $P = 0.005$)	Very limited patient number
25	McAloon	2017	260	ECM biomarkers	*n/a: meta-analysis*	n/a	Lower type I and type III collagen synthesis biomarkers (N-terminal propeptides of type I and III procollagens) predict reverse left ventricular remodelling	Meta-analysis of very different study populations
26	Dong	2011	45	Neurohormonal markers	>15% decrease in LVESV	6	Baseline PIIINP, and not the other biomarkers, was lower in CRT responders than in non-responders ($P = 0.03$); a less elevated PIIINP level in HF might be an independent biomarker predicting better response to CRT (OR 0.2, $P = 0.07$)	Very limited patient number
27	Trucco	2016	42	pTIMP1	'Clinical response, LV remodelling and mortality'	6	Baseline TIMP-1 levels are powerful predictor of long-term mortality in CRT-treated HF patients	Very limited patient number, association with mortality but not CRT response
29	Francia	2011	12	Osteopontin	Echocardiographic determinants of response to CRT	8.5 ± 4	Reverse remodelling due to CRT is reflected by changes in osteopontin	Very limited patient number, no predictive value
31	Michelucci	2016	73	Antibodies against beta1 adrenergic receptors	>15% decrease in LVESV	6	*Retrospective analysis* showed a higher percentage of Patients positive for beta1-autoantibodies (57% vs. 27%, $P = 0.004$)	limited patient number
36	Schmitz	2014	207	Genetic markers	>15% decrease in LVESV	6	Four genetic variants were associated with the CRT responder phenotype at the allelic and genotypic level	No predictive value
37	Marfella	2013	81	Circulating microRNAs	LV remodelling	12	Reverse remodelling is associated with favourable changes in miRNAs that regulate cardiac fibrosis, apoptosis and hypertrophy	Limited patient number, no predictive value
38	Melman	2015	12 (test) + 61 (val.)	MicroRNA-30d	>10% increase in LVEF	6	Baseline plasma miR-30d levels are associated with response to CRT	Very limited patient number
42	Krupa	2014	51	Oxidative stress markers	Clinical and echocardiographic determinants of response to CRT	6	Plasma concentrations of MDA, CAT, SOD and GPX were reduced in CRT responders	Limited patient number, no predictive value

Continued

Table 1 *Continued*

References	First author	Publ. year	Number of CRT patients	Biomarker	Definition of CRT response	Time frame (months)	Results	Remarks
44	Ravassa	2010	57	Annexin A5	>10% decrease in LVESVi combined with >10% increase in LVEF	12	No differences in baseline AnxA5 were observed, but reverse remodelling is associated with reduction in AnxA5 levels	Limited patient number
47	Kozstin	2017	81	CT-apelin NT-pro-BNP	A *non-response* was defined as a <4% increase in LVEF	6	CT-apelin was superior to NT-pro-BNP in identifying non-responders at 6 months	Limited patient number, no predictive value at baseline
50	Yamamoto	2013	117	Cystatin C	n/a	38, 4 (median)	Cystatin C independently predicts cardiac mortality or morbidity in patients receiving CRT	No information regarding the CRT response
51	Chatterjee	2016	133	Cystatin C	Clinical response to CRT (NYHA)	6	Cystatin C demonstrated incremental benefit in the prediction of CRT non-response *compared to standard metrics of renal function*	–
55	Maass	2018	240	15 different biomarkers	Reduction of LVESVi as a continuous variable	6	Some biomarkers were univariately associated with the CRT response, but not in the final multivariate model	–

and the incidence of a non-response [odds ratio (OR) 1.499, $P = 0.011$]. In addition, the strongest predictive factor for cardiac death was an elevated hsCRP (HR 1.337, $P = 0.001$), with a cut-off of 3 mg/L.[16] In another small pilot study (36 CRT patients) where special focus was attributed to extracellular matrix elements, CRP decreased following successful resynchronization therapy.[17]

Complement C3A

Another intriguing parameter is the amount of activated complement C3 (C3a) as a marker of chronic inflammatory state. One study investigated prospectively several components of the complement cascade (total C3, C3a, sC5b-9) in 126 HF patients, at baseline and 6 months after CRT implantation. Strikingly, CRT reduced the C3a levels (and sC5b-9 levels), and measuring C3A allowed to predict 5 years of mortality of the patients (C3a level >165 ng/mL aligned with an HR of 4.21, 95% CI 1.65–10.72, $P = 0.003$), and this effect appeared to be independent of the NT-pro-BNP levels that were measured simultaneously.[18]

Blood cell-derived parameters

Since a low lymphocytic count and high neutrophil count in haemograms of patients with HF are associated with a dismal prognosis in chronic HF, these parameters have been investigated in the CRT population. One of the main explanations for these higher neutrophil counts are an increased activation of the innate immune system in HF, and an increased neutrophil life span (viability) in HF patients.[19] In a large observational study by Boros *et al.*,[20] qualitative blood counts and NT-pro-BNP were analysed, with 2 years of mortality as primary endpoint and reverse remodelling at 6 months (>15% decrease in LVESV) as secondary endpoint. A neutrophil/lymphocyte ratio over 2.95 predicted the absence of reverse remodelling (OR 0.38 with 95% CI 0.17–0.85) and 2 years of mortality, independently of NT-pro-BNP.[20] Another, retrospective, analysis demonstrated the same finding, namely that baseline neutrophil/lymphocyte ratio was significantly higher in non-responders. In this study, patients having a ratio >3.45 had a 12-fold increased risk of CRT non-response.[20] The latter study also investigated platelet to lymphocyte ratio and percentage of lymphocytes, but these values had less predictive value.

Other inflammatory markers

Markers that sit on horseback of both inflammation, fibrosis, and remodelling are of considerable interest because they grasp important parts of the pathophysiological alterations in HF. Therefore, interleukin (IL)-6, IL-1α, IL-1β, IL-4 and IL-13, epidermal growth factor, and fibroblast growth factor 2 (FGF-2) were investigated in the multicentre Inflammatory Mediators and Clinical Outcome in Patients with Advanced Heart Failure Receiving CRT (RISK) study.[21] On multivariate analysis of 257 patients, two markers were particularly regulated. Interleukin-13 was significantly associated with the primary outcome, a combination of freedom of HF hospitalizations, death, and decrease in LVESV of >15% at 12 months' of follow-up. Detectable vs. non-detectable IL-13 levels were associated with an OR of 3.79 (95% CI 2.10–6.82, $P = 0.0001$). Conversely, detectable FGF-2 levels were negatively associated with the primary endpoint (OR 0.31, 95% CI 0.14–0.68; $P = 0.004$).[21] In the Brouwers study[7] described earlier, only lower tumour necrosis factor (TNF)-α levels were associated with a subjective response to CRT (and not with the

Table 2 Overview of the biomarkers and their mechanism of action discussed in this review

Biomarker	Abbreviation	Mechanism of action	Therapeutic role of the biomarker
Brain-derived natriuretic peptide	BNP	Markers of increased cardiac wall stress, functions as a hormone inducing natriuresis, diuresis, vasorelaxation, inhibition, or the RAAS system	Sacubitril interferes with BNP metabolism
Amino-terminal-pro-brain-derived natriuretic peptide	NT-pro-BNP		
High-sensitivity troponin T	hsTnT	Part of the troponin complex, located in the thin filament of cardiac muscle cells, regulating muscle contraction in intracellular calcium ion transportation	–
Soluble suppressor of tumorigenicity 2	sST2	functions as a 'decoy' receptor for interleukin 33, inhibiting IL-33/ST2 signalling	–
Galectin-3	Gal-3	Plays a role in numerous cellular functions including apoptosis, innate immunity, cell adhesion and T-cell regulation	–
High-sensitivity C-reactive protein	hsCRP	Belongs to the pentaxin family and is involved in several host defense related functions	–
Complement C3a	C3a	Modulated inflammation as a proteolytically processed alpha subunit	Possesses antimicrobial activity
Neutrophil/lymphocyte ratio	–	–	–
Interleukin-13	IL-13	Immunoregulatory cytokine produced by activated Th2 cells, involved in B-cell maturation and differentiation	Critical for the pathogenesis of allergen-induced asthma
Fibroblast growth factor 2	FGF-2	Cytokine having broad mitogenic and angiogenic activity, implicated in limb and nervous system development, wound healing and tumour growth	–
Interleukin-6	IL-6	Cytokine having an important function in inflammation and maturation of B cells, capable of inducing fever in autoimmune disease or infection	Anti-IL-6 agents are antirheumatic agents
Tumour necrosis factor alpha	TNF-alpha	Proinflammatory cytokine secreted by macrophages	Anti-TNF agents are anti-inflammatory agents
Cardiotrophin 1	CT-1	Cytokine inducing cardiac myocyte hypertrophy in vitro	–
N-terminal propeptides of Type I and III procollagens	PINP PIIINP	collagen precursor	–
type I collagen telopeptide	PICP	Carboxyterminal collagen crosslink, a byproduct of collagen synthesis	–
Matrix metalloproteinase 1	MMP-1	Enzyme involved in the breakdown of the extracellular matrix (breakdown of collagen)	–
Tissue inhibitor of matrix metalloproteinase 1	TIMP-1	Natural inhibitor of matrix metalloproteinases	–
Transforming growth factor beta1	TGF-beta1	Secreted ligand of TGF-beta1 superfamily, binding to TGF-beta receptors, finally regulating cell proliferation, differentiation and growth	–
Osteopontin	–	Involved in the attachment of osteoclasts to the mineralized bone matrix	–
Intermedin	–	Member of the calcitonin gene-related peptide family of hormones that play a role in the regulation of cardiovascular homeostasis, prolactin release, anti-diuresis, anti-natriuresis and regulation of food and water intake	–
Beta1 adrenergic receptor antibodies	–	Adrenergic receptors are a prototypic family of guanine nucleotide binding regulatory protein-coupled receptors that mediate the physiological effects of epinephrine and norepinephrine	Therapeutic target of beta-blocking agents
MicroRNA-30 cluster	miR-30d	–	–
Annexin A5	–	Phospholipase A2 and protein kinase C inhibitory protein with calcium channel activity and a potential role in cellular signal transduction, inflammation, growth and differentiation	–

Continued

Table 2 *Continued*

Biomarker	Abbreviation	Mechanism of action	Therapeutic role of the biomarker
CT-apelin	–	Endogenous ligand for the G-protein-coupled apelin receptor, regulating fluid homeostasis, cardiovascular function, and insulin secretion	–
Cystatin C	CysC	Extracellular inhibitor of cysteine proteases	–

objective response), in contrast to any other investigated marker [CRP, IL-6, soluble tumor necrosis factor receptor (sTNFr)1, and sTNFr2]. In a smaller study involving 46 CRT responders and 35 non-responders without any relevant baseline differences, concentrations of IL-6 and TNF-α significantly decreased in the responders to CRT.[22] And finally, levels of cardiotrophin-1 (CT-1), a member of the IL-6 family, significantly decreased in 15 CRT non-responders vs. 29 responders in a pilot study.[23] In a multivariate analysis, baseline CT-1 appeared as an independent predictor of CRT response, at least following echocardiographic standards (>15% decrease of LVESV) (OR 2.7, 95% CI 1.4–4.3; $P = 0.01$).[23]

The extracellular matrix

One of the most important features of a beneficial CRT response, especially in the super-responders to CRT, is impactful reverse remodelling of the left ventricle. This process involves significant improvement of contractile function, thickening of the walls to again physiological properties, and a decrease of the LVESV.[24] A systematic review on extracellular matrix biomarkers has been published recently.[25] N-terminal propeptides of type I and type III procollagens (PINP and PIIINP), type I collagen telopeptide (ICTP), and matrix metalloproteinase 1 (MMP-1) were measured in a subpopulation of the CARE-HF trial patient group (260 patients). In a multivariate model, these markers did not predict a CRT response, although some of them were associated with long-term cardiovascular outcomes.[13,26] Similar findings were obtained for MMP-1 and tissue inhibitor of metalloproteinase 1 (TIMP-I) in a pilot study involving 42 patients.[27] In another small pilot study ($n = 27$ ischaemic cardiomyopathy patients of which only 15 receiving CRT), MMP-9, TIMP-1, ICTP, and carboxyterminal propeptide of type 1 procollagen (PICP) were determined before and 12 weeks after CRT implantation.[28] With respect to cardiac function parameters, the MMP-9/TIMP-1 ratio correlated positively with LVEF, but the meaning of these findings is still unclear as the patient groups are very small. In the Osmancik paper,[22] discussed earlier, tissue growth factor (TGF)-β1—a stimulator of fibrosis development—significantly decreased in CRT responders, whereas in non-responders, TGF-β1 significantly increased. In this study, TGF-β1 was a significant predictor of death during follow-up. Also osteopontin, a matrix glycoprotein required for the activation of fibroblasts upon TGF-β1 stimulation, is altered in patients who show signs of CRT-induced reverse remodelling: plasma osteopontin, higher in HF patients compared to healthy controls, decreased in CRT responders, and even increased in non-responders (pilot trial involving only 12 CRT patients).[29]

Adrenergic signalling

Intermedin (adrenomedullin-2) is a member of the calcitonin gene-related peptide (CGRP) family of hormones, which play a role in the regulation of cardiovascular homeostasis, anti-diuresis, anti-natriuresis, and prolactin release.[30] Strikingly, also auto-antibodies for β1-adrenergic and muscarinic receptors are found in HF patients throughout. In a retrospective analysis of 73 HF patients[31] (NYHA II–III–IV, LVEF <35%), who all received CRT-D therapy, those auto-antibodies were measured in CRT responders and non-responders. A significantly higher percentage of patients with β1-positivity was observed in the non-responders (57% vs. 27%; $P = 0.004$), whereas antimuscarinic antibodies were not differentially regulated. The adrenergic signalling cascade is of great importance in HF: the blunted myocardial contractile reserve is partially caused by a down-regulation of β1-adrenoreceptors in the myocardium. In a preliminary study involving a limited number of patients, the proof-of-concept was shown that successful CRT pacing resulted in a significant up-regulation of β1-adrenoreceptor gene expression in the myocardium.[32] Pezzali *et al.* showed that gene polymorphisms in beta-adrenergic receptors may influence the LV reverse remodelling after CRT, and possibly also the incidence of malignant ventricular tachyarrhythmias.[33]

Molecular alterations in cardiac resynchronization therapy responders

In 2008, an important paper was published describing for the first time in detail some of the genetic alterations that occur in CRT responders.[34] Twenty-four patients underwent left ventricular biopsy procurement prior to CRT implantation, and 17 of them underwent biopsy procurement 4 months after implantation. Molecular markers at these time points were compared to a small control group of patients with normal cardiac function undergoing coronary artery bypass grafting. Responders to CRT were defined as having an increase in NYHA Class of >1, and a relative increase in LVEF of ≥25% at 4 months. Compared to the control group, HF patients had lower LV mRNA levels of alpha-myosin heavy chain (α-MHC), β-MCH, sarcoplasmic reticulum calcium ATPase 2-alpha (SERCA2α), phospholamban (PLN), and significantly higher BNP mRNA levels. The CRT responders had an increase in α-MHC, SERCA2α, an increased α-/β-MCH ratio, an increased SERCA2α/PLN ratio, and significantly lower BNP levels, while no significant changes in molecular profile were

observed in non-responders. The results shed light on the genetic mechanisms inducing reverse remodelling, which is the underlying process resulting in a beneficial response to CRT.[35] However, these molecular markers cannot serve as useful biomarkers because procurement of LV biopsies is too invasive for this purpose.

In a large consecutive follow-up study, correlation between a CRT response and genetic variants at an allelic and genotypic level was performed.[36] A total of 207 patients out of 1421 were selected and divided between responders and non-responders, and were subsequently matched for their baseline parameters before CRT. A CRT response was defined as a decrease in LVESV of >15% at follow-up echocardiography compared to baseline. Genomic DNA, extracted from patient's blood during follow-up visit, was used to perform genotyping of a selection of genetic variants based on extensive literature search focusing on cardiovascular disease and vascular remodelling. Four genetic variants—both at allelic and genotypic level—were identified with CRT response, in the following genes: ATPIB1, guanine nucleotide-binding beta polypeptide 3 (GNB3), nuclear receptor subfamily 3 group C member 2 (NR3C2), and tumour necrosis factor superfamily member 11 (TNFSF11).[36] The further-reaching aspects of this study are however that machine learning algorithms were used to fine tune the prediction of a (non-)response, based on a combination of clinical parameters and the above-mentioned genetic variants.

Epigenetic alterations in cardiac resynchronization therapy

In the last decennium, the number of publications on epigenetic regulation in the cardiovascular field has boomed. In particular microRNAs play various roles in controlling processes of cardiac hypertrophy, fibrosis, angiogenesis, apoptosis, among others. Sardu and co-workers investigated whether LV reserve remodelling after CRT was associated with changes of circulating microRNAs in patients with dyssynchronous HF.[37] In this prospective, non-randomized trial, 84 microRNAs levels were determined in 81 patients with HF eligible for CRT, against 15 healthy controls and 60 matched non-HF patients but with concomitant diseases. In the CRT population, 55 patients displayed a beneficial response, whereas the remaining 26 patients were non-responders at 12 months. At this follow-up period, the responders displayed a differential expression pattern than the non-responders: in the former group, microRNA-26b-5p, -145-5p, -92a-3p, 30e-5p, and -29a-3p ($P < 0.01$ for all microRNAs).

In 2015, the study of Melman[38] investigated the potential of 766 plasma-derived microRNAs at baseline in 12 CRT patients, with and without subsequent echocardiographic improvement at 6 months after CRT. After this pilot phase, candidate microRNAs were validated in 61 additional patients. Higher baseline microRNA-30d levels, expressed in cardiomyocytes and released in vesicles in response to mechanical stress, appeared to be associated with a beneficial CRT response, here defined as a relative increase in LVEF ≥15%. Although these results seem promising, a large validation study of microRNA-30d as a biomarker for CRT response is currently still lacking.

From these epigenetic data, it appears that the microRNA-30 cluster, consisting of microRNA-30a, -30 b, -30c1, -30c2, -30d, and -30e is critically involved in the remodelling of the left ventricle, potentially by its important role in the extracellular matrix,[39] angiogenesis,[40] and autophagy.[41] Further large validation data are eagerly awaited to confirm or refute the role of microRNAs in predicting the response to CRT.

Biomarkers related to oxidative stress and apoptosis

Few papers have explored the diagnostic potential of oxidative stress- or apoptosis-related molecules to predict a response to CRT. In a study comprising 51 patients treated with CRT, serum malondialdehyde, catalase, superoxide dismutase, and glutathione peroxidase were all reduced, and correlated with echocardiographic parameters of systolic function.[42] Ceruloplasmin, a metalloprotein that binds copper and has ferroxidase activity, is a marker of oxidative stress and is altered shortly after implantation of CRT, although the clinical usefulness of this finding is still under investigation.[43] On the other hand, annexin A5, a protein related to cellular damage, was monitored in 57 patients with HF that received CRT, at baseline and 1 year of follow-up. No differences between annexin A5 levels at baseline were observed between responders and non-responders, but after 1 year of successful CRT, annexin A5 significantly decreased, and remained unchanged in non-responders.[44]

CT-apelin

Apelin is the endogenous ligand for a G-protein-coupled apelin receptor and is investigated as an important regulator of cardiovascular homeostasis. There are several cleaved shorter peptides (apelin-36, apelin-13, etc.) originating from the 77-amino acid preproapelin, and these shorter peptides are grouped as carboxyterminal apelin fragments (CT-apelin).[45,46] In 81 patients with severe HF and implantation of a CRT device, CT-apelin was measured at baseline and 6 months after implantation. A total of 18.5% were non-responders, whereas the rest were CRT responders. At baseline, there was no difference in CT-apelin levels in both groups, but after 6 months, CT-apelin was significantly lower in the responders ($P < 0.001$).[47] Based on multivariate analysis, CT-apelin was judged superior to NT-proBNP in association with responder status. However, both markers cannot be considered predictors of CRT response as there were no baseline differences.

Cystatin C

Cystatin C (CysC) is a protein encoded by the CST3 gene. All human nucleated cells produce CysC as a chain of 120 amino acids which functions as an intracellular inhibitor of lysosomal proteinases and an extracellular inhibitor of cysteine proteases. The clinical relevance of CysC stems from extensive research in kidney failure, where the marker is of superior accuracy to more conventional, established markers of renal dysfunction e.g. serum creatinine.[48] Moreover, CysC levels have been shown to coincide with worse clinical outcome and the occurrence of clinically relevant events in patients with HF.[49] This observation lead to the hypothesis that CysC might be of

interest to predict adverse, or beneficial, outcome in HF patients whom receive resynchronization therapy. The first report on the relevance of CysC levels in predicting outcome in CRT patients was published in 2013.[50] Yamamoto *et al.* showed that elevated CysC levels were significantly associated with long-term outcome (mortality and cardiovascular events) in 117 patients with a median follow-up time of 3.2 years and an incidence rate of 29.1% for mortality, and 50.4% for cardiovascular events. This finding remained consistent even after multivariate Cox regression analysis. However, there was no superiority of CysC compared to the glomerular filtration rate (eGFR) to predict the response to CRT.[50] A few years later, in the BIOCRT study,[51] CysC levels were measured in 133 patients, both in peripheral venous and coronary sinus (CS) blood samples. Classical serum creatinine levels and eGFR, were measured simultaneously. The three markers (CysC, creatinine, and eGFR) were predictive of major adverse cardiac events (MACEs) during a follow-up term of on average 2 years, but only baseline CysC levels were associated with the identification of adverse clinical outcome after CRT implantation. CysC levels associated with CRT non-response at 6 months with an adjusted OR of 3.6 ($P = 0.02$). Moreover, the addition of CysC to classical predictive parameters resulted in an improved prediction of CRT non-response ($P \leq 0.003$). On top of that, serial measurements of CysC resulting in absolute values of >1 mg/L were associated with a CRT non-response and a reduced 6-min walk distance, as well as 2 years of MACE ($P \leq 0.04$).[51] Of note, the measurement of CysC levels in CS blood had no added value with respect to prognostic significance, meaning that peripheral blood-derived CysC levels were sufficient to predict adverse response to CRT, a feature improving the usefulness of CysC as a biomarker. Overall, the importance of CysC in the prediction of the response to CRT relates to the fact that patients with severe kidney failure display worse outcome after device therapy.[52,53]

Clinical perspectives, limitations, and future outlook

In this review, we gave an overview of the currently available evidence on biomarkers predicting the response to CRT. How should we interpret the amount of evidence presented here? As far as the classical HF biomarkers are concerned (NT-pro-BNP, BNP, hsTnT, sST2, Gal-3), a beneficial response to CRT coincides with lower values of these molecules. This finding however does not mean that these biomarkers can predict the CRT response *before* implantation! Rather these molecules reflect a beneficial reverse remodelling process after implantation. With respect to the biomarkers related to inflammation (hsCRP, complement C3a), it is interesting to note that inflammation plays an important role in the remodelling process in HF. Therefore, targeting the inflammatory pathway to find predictive biomarkers for CRT, is reasonable. Of particular interest seems complement C3a, because it also brings prognostic information on mortality after CRT implantation, beyond NT-pro-BNP levels. Closely linked to inflammation is the extracellular matrix, a paramount player in the remodelling process. In general, sample sizes of the studies on ECM-related biomarkers, adrenergic signalling, and others are too small to make relevant conclusions. Interestingly, high CysC levels at baseline coincided with adverse 6-month response to CRT, making it a potentially interesting biomarker. However, until now, it is unclear whether the predictive value of CysC is preserved when adjusting for the grade of chronic kidney failure, and this should be investigated in a large patient population.

It is clear that the need to better predict the response to CRT reflects an important clinical question as many attempts to find a useful biomarker have been undertaken. On the other hand, we conclude from our literature search that not a single biomarker is currently able to better predict the CRT response, on top of already known clinical markers. First, we have to acknowledge that a lot of research is performed, which is very positive. However, outcomes of the studies discussed are rather diffuse and difficult to interpret. This is due to the fact that (i) different definitions are applied to define the CRT response, (ii) it is sometimes unclear whether a biomarker *predicts* the response to CRT, vs. *coincides* with a beneficial CRT response, and (iii) sample sizes are generally quite small, except for some larger cohorts. Moreover, most of the papers discussed are to some extent descriptive and hypothesis-generating, rather than systematically testing or validating a predefined hypothesis. Also, most molecules are tested because they are known to be involved in the pathophysiology of HF, potentially implying some sort of selection bias.

The lack of a universal conclusion on which is the 'best' biomarker to predict the CRT response, is partly to be explained by some limitations, with which we were also confronted when listing the biomarkers studies. First, all studies used blood sampling as the option of choice to obtain human material. Related to the site of sampling, although conventional venous puncture is routinely used, CS sampling might be a favourable option.[54] This CS sampling is perfectly feasible in patients already selected for CRT, but will be hard to perform in patients who did not undergo eligibility screening. Furthermore, when sampling the CS, one should also have an idea about the cardiac output, since the transcoronary gradient is most likely not the only parameter explaining differences in biomarker levels. Moreover, the fact that CS sampling would be used is a bit in contradiction with the fact that a biomarker should be easy to procure. On the other hand, taking into account the invasiveness and the impact of the implantation procedure, CS sampling might still be acceptable in this patient group. Second, an important limitation of this review is related to the definition of a CRT response which varies significantly in the different studies in terms of the parameter(s) measured and the time point. Third, a lot of pilot data are available that did not undergo extensive validation. Fourth, the often-unclear distinction between ischaemic and non-ischaemic cardiomyopathy as the underlying pathology resulting in CRT implantation, may influence the predictive value of certain biomarkers, as the pathophysiology of non-ischaemic cardiomyopathy (NICMP) and ischemic cardiomyopathy (ICMP) is largely different. And last but not least, a careful distinction has to be made between studies investigating a single, vs. multiple, biomarkers. With respect to the latter drawback, the MARC study[55] nicely showed one of the problems with biomarker research: several of 16 investigated biomarkers were independently associated with the CRT response, but their significance diminished in a multivariate analysis.

Conclusions

Different promising candidate biomarkers have arisen that could potentially do the job, albeit maybe in a 'signature panel'. However, large

prospective patient cohorts need to be studied and several biomarkers would need to be investigated simultaneously, in order to have a reliable head-to-head comparison. Therefore, important challenges are ahead of us in order to complete the endeavour of finding the optimal biomarker to predict the response to CRT.

Conflict of interest: A.A. is a consultant to Medtronic, Boston Scientific, Biosense Webster and LivaNova. He has received speaker's fees from Medtronic, Boston Scientific and LivaNova. W.A.H. received educational support from Boston Scientific and is a consultant to Novartis and Boston Scientific. M.V. reports that he does not have conflicts of interest to disclose.

References

1. Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JG, Coats AJ *et al*. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: the Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC) developed with the special contribution of the Heart Failure Association (HFA) of the ESC. *Eur Heart J* 2016;**37**:2129–200.
2. Brugada J, Delnoy PP, Brachmann J, Reynolds D, Padeletti L, Noelker G *et al*. Contractility sensor-guided optimization of cardiac resynchronization therapy: results from the RESPOND-CRT trial. *Eur Heart J* 2017;**38**:730–8.
3. Brenyo A, Barsheshet A, Rao M, Huang DT, Zareba W, McNitt S *et al*. Brain natriuretic peptide and cardiac resynchronization therapy in patients with mildly symptomatic heart failure. *Circ Heart Fail* 2013;**6**:998–1004.
4. Zareba W, Klein H, Cygankiewicz I, Hall WJ, McNitt S, Brown M *et al*. Effectiveness of cardiac resynchronization therapy by QRS morphology in the Multicenter Automatic Defibrillator Implantation Trial-Cardiac Resynchronization Therapy (MADIT-CRT). *Circulation* 2011;**123**:1061–72.
5. Medina A, Moss AJ, McNitt S, Zareba W, Wang PJ, Goldenberg I. Brain natriuretic peptide and the risk of ventricular tachyarrhythmias in mildly symptomatic heart failure patients enrolled in MADIT-CRT. Heart rhythm: the official journal of the. *Heart Rhythm Soc* 2016;**13**:852–9.
6. Shalaby AA, Abraham WT, Fonarow GC, Bersohn MM, Gorcsan J 3rd, Lee LY *et al*. Association of BNP and troponin levels with outcome among cardiac resynchronization therapy recipients. *Pacing Clin Electrophysiol* 2015;**38**:581–90.
7. Brouwers C, Versteeg H, Meine M, Heijnen CJ, Kavelaars AM, Pedersen SS *et al*. Association between brain natriuretic peptide, markers of inflammation and the objective and subjective response to cardiac resynchronization therapy. *Brain Behav Immun* 2014;**40**:211–8.
8. McCarthy CP, Januzzi JL Jr. Soluble ST2 in heart failure. *Heart Fail Clin* 2018;**14**:41–8.
9. Skali H, Gerwien R, Meyer TE, Snider JV, Solomon SD, Stolen CM. Soluble ST2 and risk of arrhythmias, heart failure, or death in patients with mildly symptomatic heart failure: results from MADIT-CRT. *J Cardiovasc Trans Res* 2016;**9**:421–8.
10. Gehlken C, Suthahar N, Meijers WC, de Boer RA. Galectin-3 in heart failure: an update of the last 3 years. *Heart Fail Clin* 2018;**14**:75–92.
11. Stolen CM, Adourian A, Meyer TE, Stein KM, Solomon SD. Plasma galectin-3 and heart failure outcomes in MADIT-CRT (multicenter automatic defibrillator implantation trial with cardiac resynchronization therapy). *J Cardiac Fail* 2014;**20**:793–9.
12. Cleland JG, Daubert JC, Erdmann E, Freemantle N, Gras D, Kappenberger L *et al*. The effect of cardiac resynchronization on morbidity and mortality in heart failure. *N Engl J Med* 2005;**352**:1539–49.
13. Lopez-Andres N, Rossignol P, Iraqi W, Fay R, Nuee J, Ghio S *et al*. Association of galectin-3 and fibrosis markers with long-term cardiovascular outcomes in patients with heart failure, left ventricular dysfunction, and dyssynchrony: insights from the CARE-HF (Cardiac Resynchronization in Heart Failure) trial. *Eur J Heart Fail* 2012;**14**:74–81.
14. Beaudoin J, Singh JP, Szymonifka J, Zhou Q, Levine RA, Januzzi JL *et al*. Novel heart failure biomarkers predict improvement of mitral regurgitation in patients receiving cardiac resynchronization therapy—the BIOCRT study. *Can J Cardiol* 2016;**32**:1478–84.
15. Briasoulis A, Androulakis E, Christophides T, Tousoulis D. The role of inflammation and cell death in the pathogenesis, progression and treatment of heart failure. *Heart Fail Rev* 2016;**21**:169–76.
16. Kamioka M, Suzuki H, Yamada S, Kamiyama Y, Saitoh S, Takeishi Y. High sensitivity C-reactive protein predicts nonresponders and cardiac deaths in severe heart failure patients after CRT implantation. *Int Heart J* 2012;**53**:306–12.
17. Marin F, Roldan V, Martinez JG, Hernandez-Madrid A, Hernandez-Romero D, Ortego M *et al*. Influence of cardiac resynchronization therapy on indices of inflammation, the prothrombotic state and tissue remodeling in systolic heart failure: a pilot study. *Thromb Res* 2011;**128**:391–4.
18. Szeplaki G, Boros AM, Szilagyi S, Osztheimer I, Jenei Z, Kosztin A *et al*. Complement C3a predicts outcome in cardiac resynchronization therapy of heart failure. *Inflamm Res* 2016;**65**:933–40.
19. Mann DL. The emerging role of innate immunity in the heart and vascular system: for whom the cell tolls. *Circ Res* 2011;**108**:1133–45.
20. Boros AM, Szeplaki G, Perge P, Jenei Z, Bagyura Z, Zima E *et al*. The ratio of the neutrophil leucocytes to the lymphocytes predicts the outcome after cardiac resynchronization therapy. *Europace* 2016;**18**:747–54.
21. Belperio J, Horwich T, Abraham WT, Fonarow GC, Gorcsan J 3rd, Bersohn MM *et al*. Inflammatory mediators and clinical outcome in patients with advanced heart failure receiving cardiac resynchronization therapy. *Am J Cardiol* 2016;**117**:617–25.
22. Osmancik P, Herman D, Stros P, Linkova H, Vondrak K, Paskova E. Changes and prognostic impact of apoptotic and inflammatory cytokines in patients treated with cardiac resynchronization therapy. *Cardiology* 2013;**124**:190–8.
23. Limongelli G, Roselli T, Pacileo G, Calabro P, Maddaloni V, Masarone D *et al*. Effect of cardiac resynchronization therapy on cardiotrophin-1 circulating levels in patients with heart failure. *Intern Emerg Med* 2014;**9**:43–50.
24. Kim GH, Uriel N, Burkhoff D. Reverse remodelling and myocardial recovery in heart failure. *Nat Rev Cardiol* 2018;**15**:83–96.
25. McAloon CJ, Ali D, Hamborg T, Banerjee P, O'Hare P, Randeva H *et al*. Extracellular cardiac matrix biomarkers in patients with reduced ejection fraction heart failure as predictors of response to cardiac resynchronisation therapy: a systematic review. *Open Heart* 2017;**4**:e000639.
26. Dong YX, Burnett JC Jr, Chen HH, Sandberg S, Yang YZ, Zhang Y *et al*. Effect of cardiac resynchronization therapy on broad neurohormone biomarkers in heart failure. *J Interv Card Electrophysiol* 2011;**30**:241–9.
27. Trucco E, Tolosana JM, Castel MA, Batlle M, Borras R, Sitges M *et al*. Plasma tissue inhibitor of matrix metalloproteinase-1 a predictor of long-term mortality in patients treated with cardiac resynchronization therapy. *Europace* 2016;**18**:232–7.
28. Li M, Zhou Y, Zhou Y, Babu K, Wang Y. Improvement in collagen metabolism after 12 weeks' cardiac resynchronization therapy in patients with ischaemic cardiomyopathy. *J Int Med Res* 2013;**41**:200–7.
29. Francia P, Balla C, Ricotta A, Uccellini A, Frattari A, Modestino A *et al*. Plasma osteopontin reveals left ventricular reverse remodelling following cardiac resynchronization therapy in heart failure. *Int J Cardiol* 2011;**153**:306–10.
30. Zhang SY, Xu MJ, Wang X. Adrenomedullin 2/intermedin: a putative drug candidate for treatment of cardiometabolic diseases. *Br J Pharmacol* 2018;**175**:1230–40.
31. Michelucci A, D'Elios MM, Sticchi E, Pieragnoli P, Ricciardi G, Fatini C *et al*. Autoantibodies against beta1-adrenergic receptors: response to cardiac resynchronization therapy and renal function. *Pacing Clin Electrophysiol* 2016;**39**:65–72.
32. Vanderheyden M, Mullens W, Delrue L, Goethals M, Verstreken S, Wijns W *et al*. Endomyocardial upregulation of beta1 adrenoreceptor gene expression and myocardial contractile reserve following cardiac resynchronization therapy. *J Cardiac Fail* 2008;**14**:172–8.
33. Pezzali N, Curnis A, Specchia C, Carubelli V, Covolo L, Donato F *et al*. Adrenergic receptor gene polymorphism and left ventricular reverse remodelling after cardiac resynchronization therapy: preliminary results. *Europace* 2013;**15**:1475–81.
34. Vanderheyden M, Mullens W, Delrue L, Goethals M, de Bruyne B, Wijns W *et al*. Myocardial gene expression in heart failure patients treated with cardiac resynchronization therapy responders versus nonresponders. *J Am Coll Cardiol* 2008;**51**:129–36.
35. Chakir K, Daya SK, Aiba T, Tunin RS, Dimaano VL, Abraham TP *et al*. Mechanisms of enhanced beta-adrenergic reserve from cardiac resynchronization therapy. *Circulation* 2009;**119**:1231–40.
36. Schmitz B, De Maria R, Gatsios D, Chrysanthakopoulou T, Landolina M, Gasparini M *et al*. Identification of genetic markers for treatment success in heart failure patients: insight from cardiac resynchronization therapy. *Circ Cardiovasc Genet* 2014;**7**:760–70.
37. Marfella R, Di Filippo C, Potenza N, Sardu C, Rizzo MR, Siniscalchi M *et al*. Circulating microRNA changes in heart failure patients treated with cardiac resynchronization therapy: responders vs. non-responders. *Eur J Heart Fail* 2013;**15**:1277–88.
38. Melman YF, Shah R, Danielson K, Xiao J, Simonson B, Barth A *et al*. Circulating microRNA-30d is associated with response to cardiac resynchronization therapy in heart failure and regulates cardiomyocyte apoptosis: a translational pilot study. *Circulation* 2015;**131**:2202–16.
39. Duisters RF, Tijsen AJ, Schroen B, Leenders JJ, Lentink V, van der Made I *et al*. miR-133 and miR-30 regulate connective tissue growth factor: implications for a role of microRNAs in myocardial matrix remodeling. *Circ Res* 2009;**104**:170–8, 6p following 8.

40. Duan Q, Chen C, Yang L, Li N, Gong W, Li S *et al*. MicroRNA regulation of unfolded protein response transcription factor XBP1 in the progression of cardiac hypertrophy and heart failure in vivo. *J Transl Med* 2015;**13**:363–73.
41. Pan W, Zhong Y, Cheng C, Liu B, Wang L, Li A *et al*. MiR-30-regulated autophagy mediates angiotensin II-induced myocardial hypertrophy. *PLoS One* 2013;**8**: e53950.
42. Krupa W, Rozwodowska M, Sielski S, Czarnecka-Żaba E, Fabiszak T, Drewa G *et al*. Influence of cardiac resynchronization therapy on oxidative stress markers in patients with chronic heart failure. *Cardiol J* 2014;**21**:576–82.
43. Vondrakova D, Malek F, Ostadal P, Vranova J, Sedlackova L, Sediva L *et al*. Short term effect of CRT on biomarkers of cardiac remodelling and fibrosis: NT-proBNP, sST2, galectin-3, and a marker of oxidative stress–ceruloplasmin—a pilot study. *Int J Cardiol* 2012;**159**:159–60.
44. Ravassa S, Garcia-Bolao I, Zudaire A, Macias A, Gavira JJ, Beaumont J *et al*. Cardiac resynchronization therapy-induced left ventricular reverse remodelling is associated with reduced plasma annexin A5. *Cardiovasc Res* 2010;**88**:304–13.
45. Lu L, Wu D, Li L, Chen L. Apelin/APJ system: a bifunctional target for cardiac hypertrophy. *Int J Cardiol* 2017;**230**:164–70.
46. Chen Z, Wu D, Li L, Chen L. Apelin/APJ system: a novel therapeutic target for myocardial ischemia/reperfusion injury. *DNA Cell Biol* 2016;**35**:766–75.
47. Kosztin A, Szeplaki G, Kovacs A, Foldes G, Szokodi I, Vivien Nagy K *et al*. Impact of CT-apelin and NT-proBNP on identifying non-responders to cardiac resynchronization therapy. *Biomarkers* 2017;**22**:279–86.
48. Laterza OF, Price CP, Scott MG. Cystatin C: an improved estimator of glomerular filtration rate? *Clin Chem* 2002;**48**:699–707.
49. Dupont M, Wu Y, Hazen SL, Tang WH. Cystatin C identifies patients with stable chronic heart failure at increased risk for adverse cardiovascular events. *Circ Heart Fail* 2012;**5**:602–9.
50. Yamamoto T, Shimano M, Inden Y, Miyata S, Inoue Y, Yoshida N *et al*. Cystatin C as a predictor of mortality and cardiovascular morbidity after cardiac resynchronization therapy. *Circ J* 2013;**77**:2751–6.
51. Chatterjee NA, Singh JP, Szymonifka J, Deano RC, Thai WE, Wai B *et al*. Incremental value of cystatin C over conventional renal metrics for predicting clinical response and outcomes in cardiac resynchronization therapy: the BIOCRT study. *Int J Cardiol* 2016;**205**:43–9.
52. Cuculich PS, Sanchez JM, Kerzner R, Greenberg SL, Sengupta J, Chen J *et al*. Poor prognosis for patients with chronic kidney disease despite ICD therapy for the primary prevention of sudden death. *Pacing Clin Electrophysiol* 2007;**30**:207–13.
53. Hreybe H, Razak E, Saba S. Effect of end-stage renal failure and hemodialysis on mortality rates in implantable cardioverter-defibrillator recipients. *Pacing Clin Electrophysiol* 2007;**30**:1091–5.
54. Truong QA, Januzzi JL, Szymonifka J, Thai WE, Wai B, Lavender Z *et al*. Coronary sinus biomarker sampling compared to peripheral venous blood for predicting outcomes in patients with severe heart failure undergoing cardiac resynchronization therapy: the BIOCRT study. *Heart Rhythm* 2014;**11**: 2167–75.
55. Maass AH, Vernooy K, Wijers SC, van 't Sant J, Cramer MJ, Meine M *et al*. Refining success of cardiac resynchronization therapy using a simple score predicting the amount of reverse ventricular remodelling: results from the Markers and Response to CRT (MARC) study. *Europace* 2018;**20**:e1–10.